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Development of sustained release hydrophobic emulgel for oral care

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ABSTRACT

The objective of the study is to formulate the emulgel of Doxycycline hyclate and Eugenol for sustained or controlled release gel for oral care. Doxycycline hyclate is an antibiotic which belongs to chemical class tetracyclines and widely used in treatment of oral care bacterial infections. Eugenol oil is extracted from the clove and act as good analgesic and anti-inflammatory. The combination of Doxycycline hyclate and eugenol can be formulated for better oral care treatment. The compatibility study performed of drug and drug-polymer by IR spectra indicates good compatibility. The simple manufacturing method is employed as hot emulsification. The formulations were evaluated for description, pH, viscosity, spreadability, extrudability, % drug content and content uniformity. Optimized formulations were evaluated for In-vitro release, antibacterial activity, and stability study. Formulated formulations were complying with all the evaluation parameters. The highest drug release found with the formulation OFEG 01 and OFEG 06. The formulations OFEG 06 is the most stable formulation in stability at accelerated, intermediate and room temperature storage condition for 3months.

Keywords: Emulgel, Oral Care, Doxycycline and Eugenol

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INTRODUCTION

Doxycycline hyclate belongs to the class of tetracycline antibiotics which is widely to treat adult gum disease (periodontitis). Doxycycline is broad spectrum antibacterial drug widely effective in treatment of gram positive as well as gram negative bacteria. Doxycycline is indicated for use in respiratory tract infections caused by Mycoplasma neumoniae, Haemophilus influenzae, Streptococcus pneumoniae, Legionella spp., or lebsiella spp. It is also used for prophylaxis of malaria. [1-4]

Clove and clove oil are used anciently in Ayurveda as analgesic and anti-inflammatory. Eugenol is major component of clove oil. Eugenol is used in perfumeries, flavourings, essential oils and in medicine as an analgesic, anti-inflammatory, antimicrobial, and aesthetic. Eugenol is a member of the phenylpropanoids class of chemical compounds. It is a clear to pale yellow oily liquid extracted from certain essential oils especially from clove oil, nutmeg, cinnamon, basil and bay leaf.[5-8]

Periodontitis is disease condition which is characterized by inflammation of the gums and supporting structures of the teeth. Periodontitis is most widely occurring human disease. Oral drug delivery is used for sustained release and controlled release drug for better absorption and therapeutic efficacy. The sustained and controlled release of doxycycline and eugenol formulation for local application can be formulated for better treatment for longer duration. The hydrophobic formulation will remain intact in mouth for longer time due to less aqueous solubility. [9-12]

Combination of Doxycycline and eugenol will be the good therapeutic combination in same dosage form for oral care. Doxycycline will help in treatment of periodontitis and eugenol will act as an analgesic, anti-inflammatory, antimicrobial, and aesthetic. The Doxycycline will be in aqueous layer of formulation and Eugenol will be in oil phase.

MATERIAL AND METHOD

Material:

Doxycycline hyclate (Changzhou Pharmaceutical Factory, China), Eugenol (Symrise, USA), Isopropyl Myristate (BASF India), Mineral Oil (Croda, India), Butylatedhydroxytoluene (Merck Germany) Sorbitan Stearate (Croda, India), Polysorbate 20 (Seppic, France), Emulsifying wax (Connel brothers, India), Cetyl alcohol (Fine organics, India), Carbopol 974P (Lubrizol, India), Disodium EDTA (Merck,

Germany), Benzyl alcohol (Avantor, India) and Purified water (inhouse).

Method:

Compatibility study by IR:

The IR spectra of previously dried samples of solid excipients were recorded by potassium bromide dispersion technique using 2-3 mg sample of each polymer as described for solids. For lecithin, the IR absorption spectrum was recorded with FTIR instrument (with diffused reflectance) in liquid sample cell.[13-15]

Preparation of Emulgel:

Oil Phase: Melt and mix Isopropyl myristate, Mineral oil, Sorbitan stearate, Butyl atedhydroxy toulene, Sorbitan Stearate, Emulsifying wax, Cetyl alcohol and Eugenol under stirring. Maintain the temperature at 60°C. **Aqueous Phase:** Dissolve Disodium EDTA in mixture of Purified water and Polysorbate 20 under stirring. **Drug Phase:** Dissolve Doxycycline hyclate in Aqueous phase. Heat and Maintain the temperature at 60°C. **Emulsification:** Homogenise the oil phase and drug phase for 10min at temperature at 60°C. **Cooling under Homogenization:** Cool the bulk under homogenization till temperature of bulk reached to 32-35°C. **Mixing:** Mix the bulk under stirring till temperature of the bulk reaches 28-32°C. [16-18]

Table 1: Formulation composition for Emulgel

Sr. No.	Ingredients	Formulation Number					
		EMG 01	EMG 02	EMG 03	EMG 04	EMG 05	EMG 06
1	Doxycycline hyclate	2.00	2.00	2.00	2.00	2.00	2.00
2	Eugenol	2.00	2.00	2.00	2.00	2.00	2.00
3	Isopropyl Myristate	10.00	10.00	10.00	10.00	10.00	10.00
4	Mineral Oil	10.00	10.00	10.00	10.00	10.00	10.00
5	Butylatedhydroxytoluene	0.10	0.10	0.10	0.10	0.10	0.10
6	Sorbitan Stearate	1.00	2.00	3.00	1.00	1.00	1.00
7	Polysorbate 20	3.00	2.00	1.00	3.00	3.00	3.00
8	Emulsifying wax	5.00	5.00	5.00	5.00	6.00	7.00
9	Cetyl alcohol	5.00	5.00	5.00	7.00	6.00	5.00
10	Carbopol 974P	0.50	0.50	0.50	0.50	0.50	0.50
11	Disodium EDTA	0.10	0.10	0.10	0.10	0.10	0.10
12	Benzyl alcohol	1.00	1.00	1.00	1.00	1.00	1.00
13	Purified water	60.30	60.30	60.30	58.30	58.30	58.30

Evaluation Parameter:

The emulgel formulation will be evaluated for the physical, chemical and microbiological properties. Physical properties such as color, appearance, phase separation, homogeneity, smell, taste, pH, viscosity, spreadability and extrudability. Chemical properties such as % drug content and content uniformity. Microbiological properties such as microbial limit test for aerobic and anaerobic bacterial strains. [19-21]

Description:

The formulated formulations were evaluated for colour, appearance, homogeneity, phase separation, smell and taste. [22-23]

pH:

pH of the formulation was measured with digital pH meter. The formulation was diluted with purified water to make 10% Aqueous solution. The solution was made in triplicate and was measured. Mean of all the formulation results were recorded. [24-25]

Viscosity:

The Viscosity of the formulation were measured by cone and plate Brookfield viscometer, model Cap 2000+. The sample of 100mg volume approximately were added on plate of viscometer. The method was loaded of 100rpm for 1min at temperature 30°C. The samples were run for three times and average of all three results were recorded. [26-28]

Spreadability:

The spreadability of the formulations were measured by the spreading diameter of 1 g of formulations between 2 horizontal glass plates after one minute. The standard weight applied to the upper plate was 25 g. Each formulation was measured in triplicates.[29-32]

Extrudability:

The all formulated formulations were filled laminated aluminium tube. The tubes were sealed with the help of laminated tube sealing machine by applying heat at temperature 70-80°C. The measured quantity of bulk is filled in each tube laminated aluminium tube. The filled tube was placed between two glass slides and clamped together. A specified quantity of fixed weight (500g) kept on glass slides and then cap of the tube was opened. The amount of bulk formulation was collected and weighed. The % of bulk formulation extruded was calculated and grades are allotted (+++ Excellent, ++Very Good, +Good). [33-36]

% Drug Content (Assay):

The emulgel formulation containing 50 mg of Doxycycline hyclate and 12 mg of eugenol was dissolved in 25 ml of methanol (AR). The solution was sonicated for 15 minutes to complete the dissolution of the drug. The solution was filtered using Whatman filter paper (No. 41) and final volume was made up to 100 ml using distilled water. Appropriate dilutions were made using distilled water to give the solutions containing 50 µg/ml of Doxycycline

hyclate and 12 µg/ml of Eugenol (n=3). The absorbances of these solutions were recorded at 354.0 nm (λ max) for Doxycycline hyclate and 280 nm (λ max) for Eugenol by UV spectrophotometer in spectrum mode. % Drug content of the solution was calculated. [37-40]

In-vitro Evaluation:

The in vitro drug release study from the gel formulations was studied using a modified USP paddle method on Modified USP tablet dissolution test apparatus I. A glass cylinder (2.5 cm diameter), with a cellophane membrane (M.wt. cutoff 12,000) tied to one end, was attached to the metallic drive shaft of the dissolution apparatus and immersed in 250 of saline phosphate buffer pH 7.4 maintained at temperature of 37 0 C. An accurately measured volume of formulations (1gm), was transferred to the glass tube. The shaft could rotate at constant speed of 100 rpm. Aliquots of 5 ml were withdrawn at regular time intervals of 30 minutes for 8 hours, at 24 hours and at 48 hours and replaced by an equal volume of fresh buffer. The drug content in the withdrawn samples were analysed UV spectrophotometrically at λmax of 354 nm and 280 nm for Doxycycline hyclate and Eugenol respectively. The concentration of Doxycycline hyclate and Eugenol in samples was determined from a calibration curve. [41-45]

Content Uniformity

The formulation filled in 30g aluminium laminated tube and samples were analysed from Top, Middle and Bottom for the % drug content. The samples were analysed in duplicate and mean is recorded. The mean and standard deviation calculated for top, middle and bottom readings. [46-48]

Microbial Limit Test:

The microbial limit test was performed as per the USP test. The test performed for anaerobic and aerobic bacterial count. [49-51]

Antimicrobial testing

The optimized formulation was studied for Zone of inhibition by cup plate diffusion method. Staphylococcus aureus was incubated in Nutrient agar medium for 24 hours under aerobic conditions. [52-54]

The cup plate diffusion method involves sterilization of Petri plates, seeding of medium, inoculation and incubation. The plates were sterilized in autoclave. The nutrient broth for each bacterial agar was prepared in volumetric flask and sterilized by autoclaving. Molten agar (20ml) was poured in each Petri plate. The Petri plates were cooled up to 40°C and kept solidifying. Standard cultures of microbes were poured on top of the agar in the plates. After solidifying, wells of 8mm were bored aseptically using sterile cork borer. The agar plugs were taken out carefully so as not to disturb the surrounding medium. The holes were filled with 0.1ml different gel formulations and drug solution. The plates were kept in an incubator at 37°C for specified time. After this Petri plates were observed for the antibacterial activity by measuring the zone of inhibition using scale.

RESULTS AND DISCUSSIONS

Compatibility study by IR:

The IR Spectra shows that the functional groups of Doxycycline hyclate and Eugenol were intact in mixture of Doxycycline hyclate and Eugenol, Doxycycline hyclate and polymer, and eugenol and polymer. The Doxycycline hyclate and Eugenol are compatible with each other as well as with polymers.

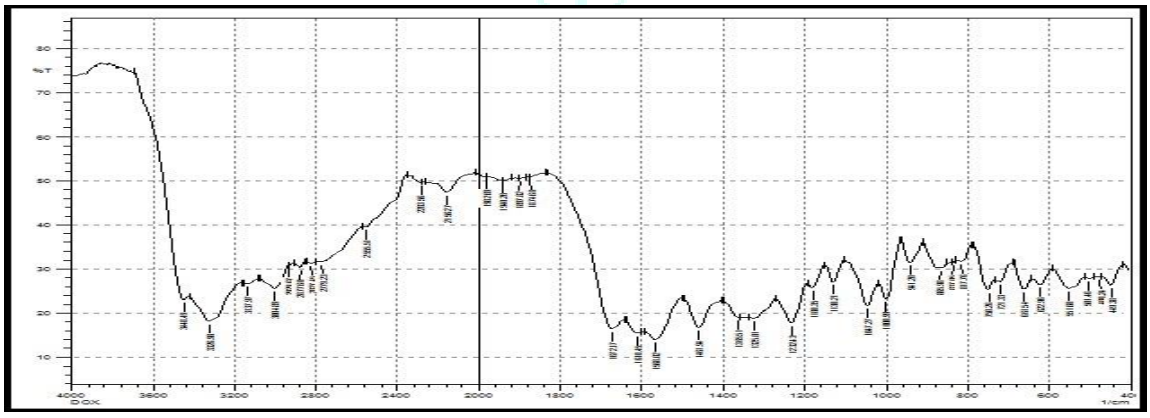

Figure 1: IR spectrum of Doxycycline hyclate

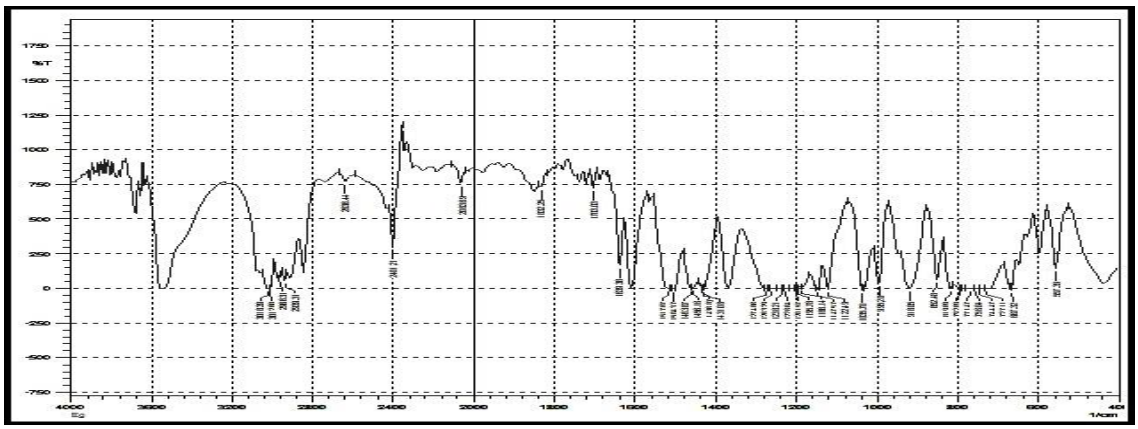

Figure 2: IR spectrum of Eugenol

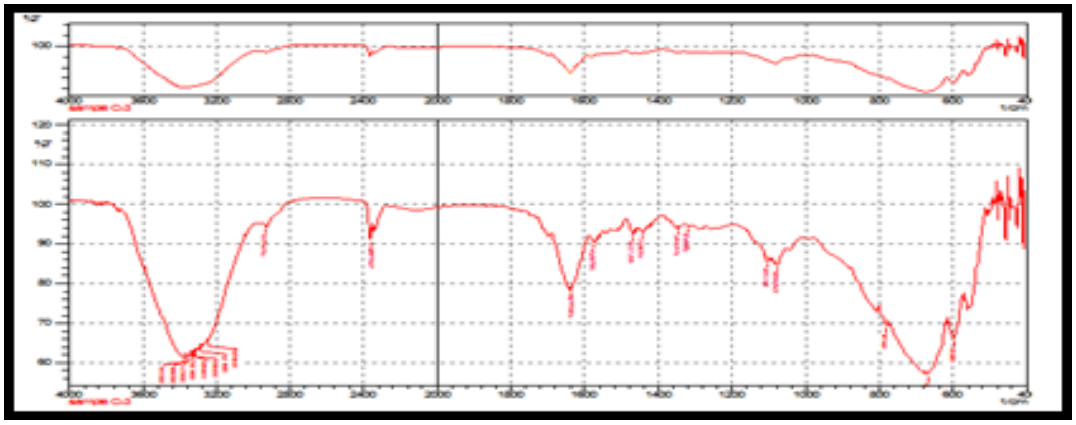

Figure 3: IR spectrum of Doxycycline hyclate and Eugenol

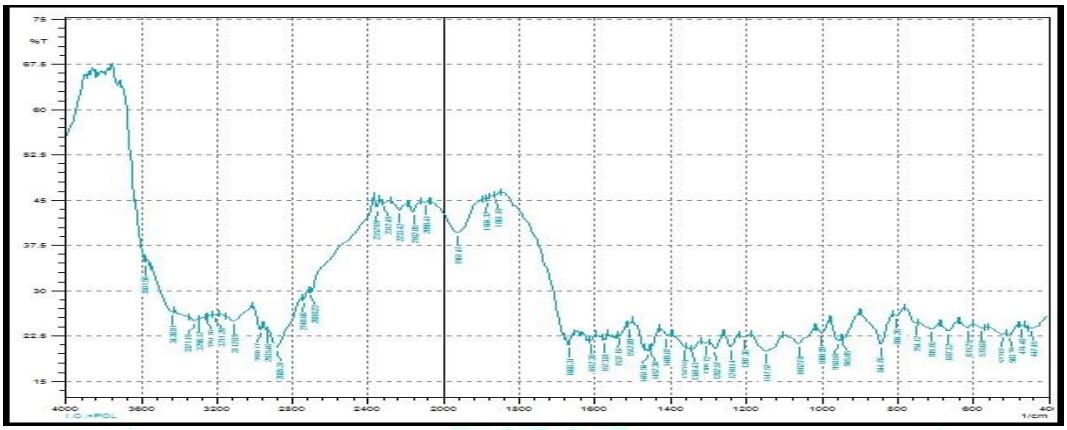

Figure 4: IR spectrum of physical mixture of Doxycycline hyclate and polymers

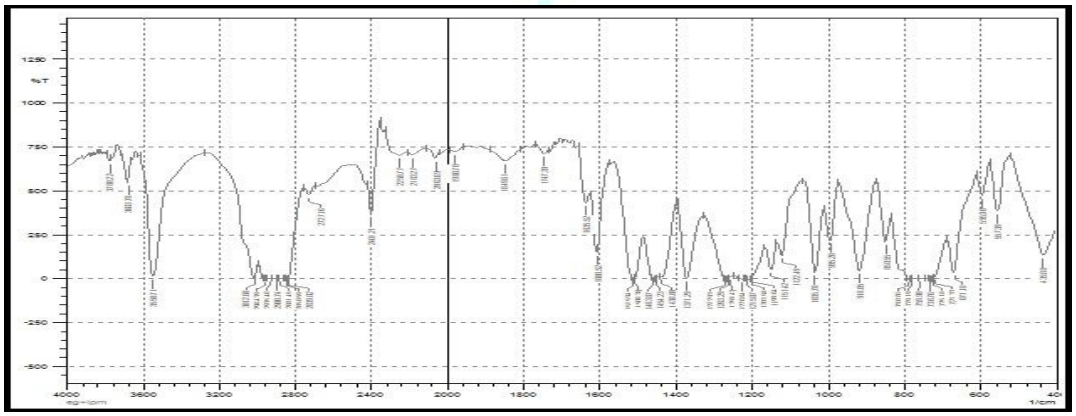

Figure 5: IR spectrum of physical mixture of Eugenol and polymers

Evaluation Parameter:

Description:

The observations were recorded for colour, appearance, homogeneity, phase separation, smell and taste in Table 2. Description of the formulation is like white smooth homogenous cream without phase separation with acceptable smell and taste.

Table 2: Results for the description of the formulations

Batch No.	Colour	Appearance	Homogeneity	Phase Separation	Smell	Taste
OFEG 01	White	Smooth shiny	Yes	No	Acceptable	Acceptable
OFEG 02	White	Smooth shiny	Yes	Yes	Acceptable	Acceptable
OFEG 03	White	Smooth shiny	Yes	Yes	Acceptable	Acceptable
OFEG 04	White	Smooth shiny	Yes	No	Acceptable	Acceptable
OFEG 05	White	Smooth shiny	Yes	No	Acceptable	Acceptable
OFEG 06	White	Smooth shiny	Yes	No	Acceptable	Acceptable

pH:

The pH of the all the formulations were stable and in range of 6.0 to 7.0. pH of the final formulation adjusted with Sodium hydroxide solution. The results are tabulated in table 3.

Table 3: Results for the pH, Viscosity, spreadability, extrudability and % drug content

Batch No.	pH	Viscosity	Spreadability	Extrudability	% Drug content
OFEG 01	6.50	4.56	14.89	+++	98.9
OFEG 02	6.67	4.13	15.11	++	86.8
OFEG 03	6.89	4.66	14.97	++	90.6
OFEG 04	6.45	6.67	12.67	+++	99.6
OFEG 05	6.47	6.01	13.56	+++	98.7
OFEG 06	6.52	5.89	14.45	+++	97.7

Viscosity:

The viscosity of the formulation was found in the range of 4.13-6.67 Cps. The results are tabulated in table 3. The viscosity of the formulation is dependant on the concentration polymer and the concentration of solid emulsifiers in formulation. The increase in the polymer concentration and solid emulsifiers concentration viscosity of the formulation increases. The solid emulsifiers are also contributing to the formation of viscosity.

Spreadability:

The spreadability of the formulation was found in the range of 12.67-15.11 g/cm^{-1}. The results are tabulated in table 3. The spreadability of the formulation is dependant on the viscosity of the formulation. Higher the viscosity of the formulation, lower will be the spreadability of the formulation.

Extrudability:

The extrudability of the formulation will be dependant on the viscosity of the formulation and smooth texture of the formulations. The extrudability of the formulation was acceptable. The results were tabulated in the Table 3. (+++ Excellent, ++Very Good, +Good).

% Drug Content (Assay):

The drug content of the formulations was found in the range 86.8-99.6%. The % drug content of all the formulation were acceptable and tabulated in table 3. The assay of the formulation OFEG 02 and OFEG 03 was found to be less, this may be due to separation of phases which lead to show variation in assay.

Content Uniformity

The content uniformity of all the formulations complies with USP specifications except formulations OFEG 02 and OFEG 03. The results are tabulated in Table 4. The container uniformity results for the formulation batch number OFEG 02 and 03 fails to comply with the specification. This may be due to phase separation of the formulation.

Table 4: Results for the content uniformity and microbial limit test

Batch No.	Content Uniformity					Microbial Limit test
	Top	Middle	Bottom	Mean	SD	
OFEG 01	98.7	99.0	98.6	98.8	0.2	Complies as per USP
OFEG 02	85.8	105.5	80.5	90.6	13.2	Complies as per USP
OFEG 03	88.6	91.5	109.7	96.6	11.4	Complies as per USP
OFEG 04	99.3	99.1	98.8	99.1	0.3	Complies as per USP
OFEG 05	98.6	98.5	98.3	98.5	0.2	Complies as per USP
OFEG 06	97.9	98.2	98.3	98.1	0.2	Complies as per USP

In-vitro Evaluation:

The invitro release of the Doxycycline was found the range of 63-77% after 48 hrs. The formulation OFEG 01 has highest drug release amongst all he formulation. This may be due to low viscosity of the formulation. The formulation OFEG 02 has much slower drug release than the other formulation. The drug release pattern of OFEG 06 and OFEG 01 is same but the OFEG 01 has higher drug release than the OFEG 06. The results are tabulated in Table 5 and graphically represented in figure 6.

Table 5: Results for the In-vitro release test for Doxycycline.

Time (min)	Doxycycline hyclate			Eugenol	
	OFEG 01	OFEG 04	OFEG 06	OFEG 01	OFEG 06
0	0.000	0.000	0.000	0.000	0.000
30	13.289±0.81	7.194±0.13	10.373±0.11	9.352±0.04	8.482±0.04
60	19.021±0.13	11.049±0.26	17.805±0.20	15.358±0.04	13.561±0.11
90	25.504±0.05	16.651±0.21	23.656±0.35	20.746±0.03	18.782±0.05
120	30.460±0.15	17.914±0.12	28.463±0.40	26.342±0.05	24.431±0.06
150	35.000±0.12	19.491±0.24	34.384±0.31	31.587±0.05	29.228±0.02
180	39.699±0.21	23.879±0.23	38.383±0.11	37.203±0.05	36.944±0.18
210	43.971±0.65	27.382±0.16	42.718±0.05	42.580±0.04	40.339±0.16
240	48.727±0.17	32.332±0.17	46.588±0.29	47.529±0.04	45.670±0.14
270	52.191±0.08	37.448±0.28	50.496±0.23	51.947±0.06	49.921±0.22
300	56.834±0.00	35.325±0.22	53.559±0.30	55.755±0.08	53.782±0.25
330	60.994±0.32	40.789±0.21	57.167±0.33	58.036±0.09	56.626±0.19
360	63.883±1.52	46.772±0.32	61.925±0.29	60.067±0.10	58.041±0.23
390	66.705±1.12	51.975±0.18	64.637±0.25	63.192±0.10	61.445±0.19
420	70.028±1.09	54.744±0.07	67.341±0.27	66.500±0.09	64.746±0.32
450	74.535±0.41	59.058±0.07	71.946±0.28	69.318±0.08	67.625±0.33
480	77.916±0.71	63.968±0.09	74.529±0.22	72.453±0.08	70.102±0.25

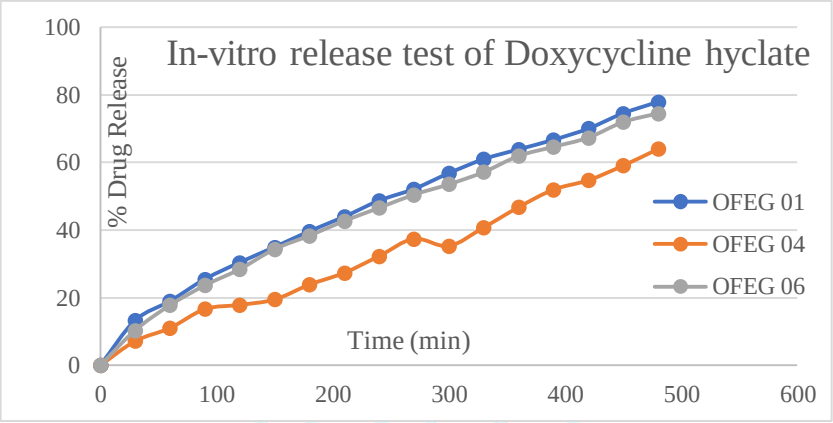

Figure 6: Comparative In-vitro drug release profile Doxycycline

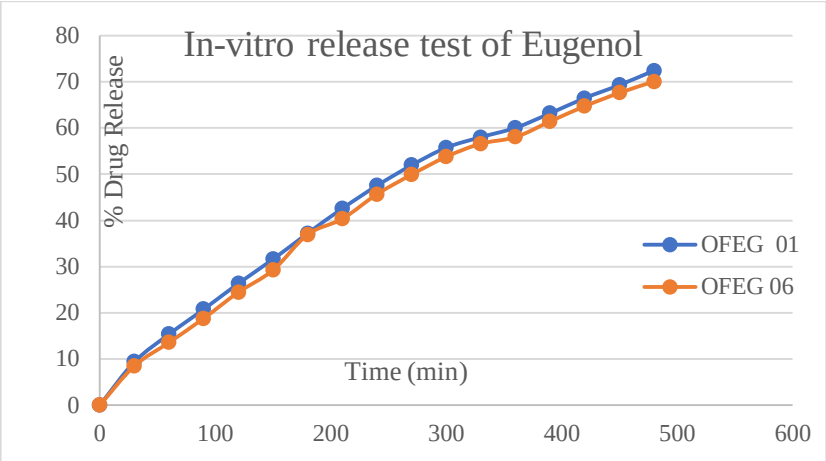

Figure 7: Comparative In-vitro drug release profile Eugenol

Antimicrobial testing

The zone of inhibition (ZOI) Formulations OFEG 01, OFEG 04 and OFEG 06 were found in range of 3.8-4.5. The results are tabulated in Table 6. The ZOI was found highest for formulation OFEG 01, this may be due to highest drug release from the formulation. The higher drug release may be due to low viscosity of the formulation.

Table 6: Results for the Antimicrobial testing.

Batch No.	OFEG 01	OFEG 02	OFEG 03	OFEG 04	OFEG 05	OFEG 06
Zone of Inhibition (cm)	4.50	4.25	4.29	3.89	3.75	4.35

CONCLUSION

The emulgel formulation can be formulated with the composition of Doxycycline hyclate, Eugenol, Isopropyl Myristate, Mineral Oil, Butylatedhydroxytoluene, Sorbitan Stearate, Polysorbate 20, Emulsifying wax, Cetyl alcohol, Carbopol 974P, Disodium EDTA, Benzyl alcohol, and Purified water. The doxycycline and eugenol are compatible with each other as well as polymer. The formulations have good stability and complies with the evaluation parameter except formulations OFEG 02 and 03. The invitro release study has shown that formulation OFEG 01 and OFEG 06 has similar pattern of drug release but formulation OFEG 04 has slower drug release. The formulation OFEG 01 and 06 has similar antimicrobial efficacy on nutrient media. The combination of Doxycycline hyclate and Eugenol has good antibacterial activity and expected to have better therapeutic efficacy. The formulation OFEG 06 has better stability at accelerated storage condition for 3Months.

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